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# Exploring the Relationship between Transport, Resistance, and Virulence Factors of Escherichia coli Collected from Swine Manure

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# Exploring the Relationship between Transport, Resistance, and Virulence Factors of *Escherichia coli* Collected from Swine Manure

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## ABSTRACT

Broad spectrum antibiotics, such as Tylosin (naturally synthesized by *Streptomyces fradiae*) are used to treat infections in farm animals and are often administered at sub-therapeutic levels along with feed rations. The presence of bacteria resistant to antimicrobials in animal waste has raised concern related to their transport to surface and groundwater. Previous studies have shown that cells preferentially attach to sediments affecting their transport in overland flow; however, a lack of quantitative understanding exists regarding the attachment mechanisms such as extracellular organelles and the relationship between these and virulence factors in humans. In a preliminary study, the presence of Tylosin resistant bacteria have been enumerated in tile drainage samples collected beneath no-till plots. While this study shows the transport potential of resistant bacteria, the method of transport, the relationships between transport and resistance, and attachment and virulence factors is unknown. The objective of this research is to study these relationships in *Escherichia coli* collected from swine manure. Cultures will be screened for antibiotic resistance, and multiple antibiotic resistances. Resistant and non-resistant cultures will be grown in a chemostat environment to mimic a low nutrient environment. Relationships will be studied *in vitro* using an attachment assay and PCR. This study will be important in determining the relationship between antibiotic resistant bacteria and their ability to move within the environment and impact human health.

## ACKNOWLEDGEMENTS

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## INTRODUCTION

In swine production, the development of a large number of antibiotics and other additives has helped increase gain and reduce the feed required per unit of gain. At least 11 antimicrobial or antifungal compounds or groups of compounds are widely used in swine feeds. These include various salts of bacitracin, chlortetracycline, dynafac, mycostatin, oxytetracycline, oleandomycin, penicillin, streptomycin, bambermycins, tilmicosin and tylosin (Carlson and Fangman, 2000). Veterinarians commonly use feed-grade antibiotics at therapeutic levels to treat acute disease outbreaks. This ensures that the bacterial pathogen causing the observed symptoms is treated with an antibiotic to which the pathogen has demonstrated susceptibility. However, in many instances, antibiotics are used to promote growth or weight gain and are fed at sub-therapeutic levels on a daily basis.

## OBJECTIVE

To demonstrate that selection for antibiotic resistance by sub-therapeutic antibiotic use in agriculture co-selects for bacteria with increased attachment to sediment and increased frequency of virulence-associated genes

## ISOLATE COLLECTION

- Manure was collected from 6 locations (Figure 1)
  - 2 Organic
    - Finish operation: 90 hogs
    - Farrow operation: 168 sows
  - 4 Sub-therapeutic
    - Farrow operation: 100 sows
    - Finish operation: 720 hogs
    - Finish operation: 4000 hogs
    - Finish operation: 4800 hogs
- *E. coli* colonies were enumerated from manure via membrane filtration
- 100 typical and atypical colonies from each location were selected for further study

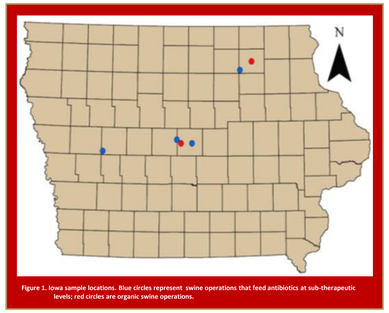


Figure 1. Iowa sample locations. Blue circles represent swine operations that feed antibiotics at sub-therapeutic levels; red circles are organic swine operations.

## PHENOTYPIC ATTACHMENT

### *Assay Preparation*

- Individual strains were grown for 12 hours in 10 mL of Mueller Hinton Broth and transferred to a phosphate buffered water solution
- Cultures were diluted to an optical density of 0.5 McFarland Standard
- 404 strains from a library of 556 strains were characterized by enumeration of total *E. coli*
- 0.4 grams of sand particles with an average diameter of 125µm were added to 40 mL of suspended cells

### *Attachment Assay*

- Cultures were mixed for 20 minutes on an orbital shaker at room temperature
- Static settling occurred for 5 minutes, which is adequate time for the smallest sand particle to settle out of suspension according to Stoke's Law
- Unattached fraction were characterized by enumeration
- Attachment phenotype was calculated as the difference between total and unattached *E. coli* for each strain (Figure 2)

### *Results*

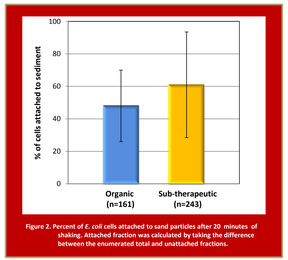


Figure 2. Percent of *E. coli* cells attached to sand particles after 20 minutes of shaking. Attached fraction was calculated by taking the difference between the enumerated total and unattached fractions.

- Attachment is statistically significant among the treatments ($p < 0.0001$)
- Pressure from the antibiotics from the sub-therapeutic operations may be responsible for increased attachment

### *Further Research*

- Further characterization of the phenotypic and genotypic attachment is needed
- Isolates will be characterized from an additional organic operation (winter 2009)

## ANTIBIOTIC RESISTANCE

### *Pre-screening*

- 70 Tylosin resistant *E. coli* colonies were collected, re-plated on agar infused with:
  - Chloramphenicol (MIC = 2 µg $L^{-1}$)
  - Ampicillin (MIC = 2 µg $L^{-1}$)
  - Erythromycin (MIC = 8 µg $L^{-1}$)
  - Tylosin (MIC = 32 µg $L^{-1}$)
- Plates were incubated at 37°C for 24 hours

## ANTIBIOTIC RESISTANCE

### *Pre-screening continued*

- Resistance was recorded as presence/absence (Figure 3)

### *Results*

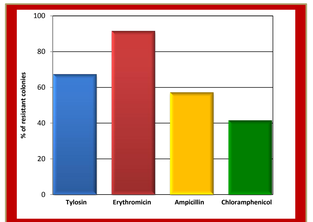


Figure 3. Percentage of resistance colonies to Tylosin, Erythromicin, Ampicillin, and Chloramphenicol. Resistance was recorded as presence/absence (n=70).

- 22.9% of the cultures exhibited resistance to all tested antibiotics
- 55.7% of cultures resistant to tylosin were resistant to > two other antibiotics

### *Further Research*

All library strains will be screened for resistance against 15 antibiotics as identified as being important to both humans and animals by NARMS (2005) and the USDA APHIS Report.

## GENOTYPE ATTACHMENT AND VIRULENCE FACTORS

- Isolates were queried by gene-specific PCR for known attachment and virulence factors (Table 1)
  - 43 isolates from organic operations
  - 43 isolates from sub-therapeutic operations
- Primers specificity was assessed by running a negative and positive control

### *Results*

Table 1. *E. coli* strains isolated from sub-therapeutic operations have increased frequency of the genes encoding P pili relative to isolates from organic operations. Values indicates the percent of the queried population that tested positive for the indicated factor. 43 strains were analyzed from each set by gene-specific PCR.

| Attachment Factor | Organic | Sub-therapeutic |
|---|---|---|
| P pili | 0 | 13.95 |
| Type 1 pili | 74.42 | 58.14 |
| S fimbriae | none detected | |
| Dr hemagluttinin | none detected | |

| Virulence Factor | Organic | Sub-therapeutic |
|---|---|---|
| hemolysin A | none detected | |
| cytotoxic necrotizing factor | none detected | |

- Increased coding frequency for P pili support our key hypothesis
- Genes encoding P pili have been previously reported as being associated with antibiotic resistance (Karami et al., 2008; Arisoy et al., 2008)

### *Further Research*

- Continue querying isolates by gene-specific PCR for known virulence and attachment factors
- Correlate attachment and antibiotic resistance phenotype with virulence and attachment factor genotype